Prevention of Edema Disease in Pigs by Vaccination with Verotoxin 2e Toxoid

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ABSTRACT

Pigs in 2 herds with persistent problems with post weaning edema disease caused by infection with verotoxin-2e (VT2e)-producing *Escherichia coli* O139 were treated with a VT2e-toxoid vaccine. Treatment was performed as a randomized blind field trial with parallel treatment and non-vaccinated control groups. In 1 herd, a group of pigs was injected with adjuvant alone. Pigs were vaccinated at 1 and 3 wk of age and weaned at 4 wk of age. The effect of vaccination was measured by average daily weight gain (ADG), mortality due to edema disease within the 1st 4 wk after weaning, and weight at 3–6 mo of age. Pathological and microbiological examinations were performed on all pigs that died during the 1st 4 wk post weaning. Only pigs from which VT2e+, F18+ *E. coli* O139 was isolated were categorized as "death due to edema disease." The serological response to vaccination was evaluated by an indirect ELISA. Vaccination had a statistically significant effect on the level of antibodies specific for VT2e in both herds. Vaccination resulted in a statistically significant increase in ADG in the nursery period but not in the grower-finishing period. Vaccination had a statistically significant effect on mortality due to edema disease with an odds ratio of 0.039, indicating that there was almost total elimination of mortality due to the disease in the vaccine groups.

RÉSUMÉ

Des porcs provenant de deux troupeaux affectés chroniquement de la maladie de l'œdème en période post-sevrage causée par un isolat de *Escherichia coli* O139 producteur de vérotoxine de type 2e (VT2e) ont été traités avec un toxoïde de VT2e. Le traitement fut effectué dans une étude randomizée menée à l'aveugle avec en parallèle un groupe traité et un groupe témoin non-vacciné. Dans un des troupeaux, un groupe d'animaux ne fut injecté qu'avec de l'adjuvant. Les porcs ont été vaccinés à 1 et 3 semaines d'âge et sevrés à 4 semaine d'âge. L'effet de la vaccination fut mesuré par la prise du gain journalier moyen, la mortalité due à la maladie de l'œdème au cours des quatre premières semaines après le sevrage, et le poids à 3–6 mois d'âge. Des examens pathologique et microbiologique furent effectués sur tous les porcs qui moururent durant les quatre premières semaines suivant le sevrage. Seuls les porcs à partir desquels on isola *E. coli* O139 VT2e+ et F18+ furent inclus dans la catégorie « mort due à maladie de l'œdème. » La réponse sérologique à la vaccination fut évaluée par une épreuve ELISA indirecte et cette dernière a permis de démontrer que la vaccination avait un effet significatif sur le niveau d'anticorps spécifiques envers VT2e dans les deux troupeaux. La vaccination entraîna également une augmentation significative du gain moyen quotidien durant la période en pouponnière. De plus, la vaccination a eu un effet statistiquement significatif sur la mortalité (« *odds ratio* » 0,039) et élimina presque complètement la mortalité due à la maladie de l'œdème dans les groupes d'animaux vaccinés.

(Traduit par docteur Serge Messier)

INTRODUCTION

Edema disease (ED) is an *Escherichia coli* enterotoxemia in pigs usually occurring in the 1st 2 wk after weaning, but older pigs and sows can also be affected (1). In Denmark the disease has been caused by hemolytic F18-positive *E. coli* O139 which produce verotoxin-2e (VT2e), also designated Shiga-like toxin IIe (SLT-IIe) (2). The clinical signs are edema of the palpebrae, neurological disorders, and sudden death (4). By gross examination, edema may be observed in the submucosa of the stomach and in the mesocolon, and by microscopic examination, lesions of edema, hemorrhage, and vasculitis are noted (4). Surviving pigs usually become unthrifty and animals with milder infections show reduced daily weight gain and feed conversion (5,6). If the *E. coli* O139 has the ability to produce heat stable enterotoxin (ST) and or heat-labile enterotoxin (LT), diarrhea is usually the dominant clinical picture in the herd (2,3).

F18 fimbriae are responsible for adhesion of *E. coli* O139 to the intestinal wall, thereby permitting colonization of the small intestine of pigs that possess the intestinal receptor for the adhesin (3). VT2e is absorbed through the intestinal wall and enters the vascular system (8). The degree of damage caused by the toxin depends on the quantity absorbed (7) and the number and distribution of receptors in the target organs (8).

Purified VT2e injected intravenously in pigs reproduces all the clinical signs and pathological lesions of ED (7) and is therefore a vaccine candidate for stimulation of protective immunity in pigs. Since VT2e is highly toxic for the pig, it cannot be

used directly for immunization of pigs against ED. Detoxification of VT2e has been attempted by treatment of toxin with formaldehyde or glutaraldehyde or by creation of specific mutations in the toxin gene (6). A mutant toxin produced by replacing glutamate at position 167 of the A subunit with glutamine was considerably reduced in toxicity and induced a high neutralizing antibody titre in pigs. Absence of lesions of ED in the pigs suggested that the mutant was safe. Subsequently, pigs inoculated with the mutant toxin were fed high protein diets and challenged with an ED strain of *E. coli* (5). These studies demonstrated that the vaccine induced protection against microscopic lesions as well as mortality due to ED. Immunization with VT2e inactivated by 1% formaldehyde has been shown to reduce weight gain because of residual toxicity (6). Awad-Masalmeh et al (9) showed that immunization with 5 mg of a bacterial lysate containing VT2e inactivated by glutaraldehyde resulted in a good protection against challenge with an ED strain of *E. coli* in experimental models. However, because of toxic effects the vaccine also resulted in a 1.2% mortality when used under field conditions.

MacLeod and Gyles showed that purified VT2e toxin inactivated by glutaraldehyde protected all vaccinated pigs against death when they were challenged in an experimental ED model (10). The study also showed that only the animals with the lowest levels of antibodies had edema of the palpebrae. However, there have been no studies in which a purified toxoid vaccine has been tested under field conditions. The objective of the present study was to treat pigs in 2 Danish herds with post weaning ED with a VT2e-toxoid vaccine.

MATERIALS AND METHODS

VACCINATION

The vaccine was an experimental toxoid vaccine based on purified glutaraldehyde inactivated VT2e (10) (25 μg/mL) with 15% Emulsigen (MVP Laboratories, Ralston, Nebraska, USA) as adjuvant. Piglets were vaccinated by the intramuscular route at 1 and 3 wk of age with 12.5 and 25 μg of inactivated toxin, respectively, and

TABLE I. Summary of the study design to evaluate effectiveness of a VT2e toxoid vaccine in 2 Danish pig herds

	Herd A	Herd B
Herd size	170 sows	300 sows
Number of sows in trial	24	35
Number of pigs in vaccine group	127	113
Number of pigs in adjuvant group	0	114
Number of pigs in control group	128	117
Weaning age	27 d (24–27)[a]	29 d (24–47)
Period in nursery	27 d (21–30)	41 d (34–48)
Age at 1st vaccination	6 d (3–9)	7 d (3–13)
Age at 2nd vaccination	20 d (17–23)	21 d (16–23)
Time of 1st blood sample	1st vaccination	1st vaccination
Time of 2nd blood sample	Weaning	Weaning
Time of 3rd blood sample	End of nursery period	End of nursery period
Time of 4th blood sample	82 d p.w.[b] (70–93)	127 d p.w. (111–148)
Time of 1st weighing	Weaning	Weaning
Time of 2nd weighing	End of nursery period	End of nursery period
Time of 3rd weighing	82 d p.w. (70–93)	127 d p.w. (111–148)
Weight at weaning	6.5 kg (1.6–11.9)[c]	8.6 kg (4.9–14.0)

[a] Mean: x̄ d (min–max)
[b] Post weaning
[c] Mean: x̄ kg (min–max)

were weaned at 4 wk. The toxin was purified by cation exchange chromatography of a polymyxin B extract of *E. coli* JM101(pGT110) carrying a cloned VT2e gene under the control of the *tac* promoter (11). The volume of material injected was 0.5 and 1.0 mL, respectively.

EXPERIMENTAL DESIGN

The treatment was performed in 2 Danish specific pathogen free herds with 170 (herd A) and 300 sows (herd B), respectively, as a randomized blind field trial with parallel treatment and control groups. Herd A and B were free from *Actinobacillus pleuropneumoniae* (all serotypes), toxin producing *Pasteurella multocida*, *Serpulina hyodysenteria* and mange, i.e. *Sarcoptes scabiei* var. *suis*. Herd A was also free from *Mycoplasma hyopneumoniae*. Before this trial both herds had persistent problems with ED in weaned pigs. The experimental design is summarized in Table I. In herd B, pigs in the adjuvant group were injected with phosphate buffered saline with 15% Emulsigen. The effect of vaccination was measured by average daily weight gain (ADG) and mortality due to ED in the nursery period. To evaluate the long term effect of vaccination on weight gain all pigs were weighed at 3–6 mo of age. Data on weight and serology from all pigs that died were omitted from the statistical analyses.

PATHOLOGICAL AND MICROBIOLOGICAL EXAMINATIONS

All piglets that died in the nursery period were sent to the Danish Veterinary Laboratory for pathological and microbiological investigations. Hemolytic *E. coli* were serotyped by slide agglutination tests and tested by PCR for the genes of the fimbriae F4, F5, F6, F18 and F41 and the genes of the toxins STa, STb and VT2e (12). Only pigs from which F18+, VT2e+ *E. coli* O139 was isolated were categorized as "death due to edema disease."

SEROLOGY

The serological response to vaccination was monitored by an indirect ELISA that was used to detect specific anti-VT2e antibodies in serum obtained from approximately 50% of the animals in each group at 4 times. Briefly, VT2e-toxoid (3 μg/mL) in 50 mM carbonate buffer, pH 9.0, was applied directly to ELISA plates (MaxiSorp, NUNC, Denmark) at 4°C over night. The plates were incubated with 0.05% Tween 20 (v/v), 1% bovine serum albumin in PBS (PBS-T-BSA) (200 μL/well) for 1 h. Serum samples were applied in 1:200 dilutions in PBS. Antibodies were detected with peroxidase-conjugated rabbit anti-swine immunoglobulins (P 0164, DAKO, Denmark) in PBS-T-BSA. A solution containing 670 μg/mL o-phenylenediamine and 0.0125% H_2O_2 dissolved in 35 mM citric acid, 67 mM Na_2HPO_4, pH 5.0 was used as

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TABLE II. Effect of vaccination on mortality, weight gain, and specific anti-VT2e antibodies in 2 pig herds

| | Herd A | | Herd B | | |
Mortality % (number of pigs)	Vaccine	Control	Vaccine	Adjuvant	Control
Edema disease	0.8 (1)	6.3 (8)	0.0 (0)	7.0 (8)	10.6 (12)
Other causes	7.9 (10)	8.6 (11)	3.4 (4)	13.2 (11 + 4)[a]	16.8 (14 + 5)[b]
Average daily gain (g/d)					
Nursery	301 (86)[c]	279 (94)	382 (100)	320 (113)	316 (121)
Grower and finisher	423 (103)	429 (78)	766 (105)	757 (147)	735 (92)
Whole period	383 (77)	380 (71)	643 (82)	616 (107)	604 (78)
Serology (OD values)					
Sow, serum	0.34 (0.32)[c]	0.32 (0.27)	0.18 (0.05)	0.18 (0.04)	0.17 (0.03)
Sow, colostrum	0.51 (0.18)	0.51 (0.18)	0.45 (0.23)	0.48 (0.24)	0.47 (0.26)
Pigs, before 1st vaccination	0.21 (0.07)	0.23 (0.08)	0.24 (0.07)	0.24 (0.09)	0.24 (0.09)
Pigs, at weaning	1.10 (0.73)	0.13 (0.11)	0.74 (0.43)	0.11 (0.04)	0.11 (0.03)
Pigs, end of nursery period	1.71 (1.21)	0.36 (0.77)	1.33 (0.76)	0.17 (0.07)	0.19 (0.14)
Pigs, 10–14 wk p.w.	0.75 (0.51)	0.29 (0.19)	0.86 (0.34)	0.23 (0.08)	0.23 (0.06)

[a] Four pigs had signs of clinical edema disease but no microbiological confirmation
[b] Five pigs had signs of clinical edema disease but no microbiological confirmation
[c] $\bar{x}$ (standard deviation (SD))

substrate (100 μL/well). Colour was allowed to develop for 20 min and the reaction was stopped by addition of 0.5 M sulphuric acid (150 μL/well). The optical density (OD) was measured at 490 nm minus 650 nm by dual wavelength endpoint read in an ELISA plate reader.

STATISTICAL ANALYSIS

The possible treatment effect was expected to be largest on mortality due to ED, and this was tested by a logistic regression model. Herd B was analyzed including different time aspects such as the age of the piglets at the time of vaccination. Due to this exploratory study the final model was:

$$log \frac{\pi_{ijkl}}{1 - \pi_{ijkl}} = \mu + \tau_i + \zeta \cdot w_{l(k(j))} + H_j + S_{k(j)}$$

where π_{ijkl} was the proportion of pigs dying from ED, while τ_i was the treatment having three levels control, adjuvant, and vaccinated. The weight of the pig at weaning was designated by $w_{l(k(j))}$ and ζ the parameter reflecting the actual influence on the logit. Two random components were added, H_j due to herd and $S_{k(j)}$ related to the sows. The distribution of these components was assumed to be normal, with a mean of zero and variances of σ_H^2 and σ_S^2 respectively.

Analyses of weight gain and weight were performed separately for the 2 herds by linear regression models. The daily weight gain from weaning to the end of the nursery period was analyzed by the model:

$$w_{ijklm} = \mu + \tau_i + \zeta \cdot x_{l(k(j))} + \gamma_m + G_j + S_{k(j)} + \tau_i S_{k(j)}$$

where w denoted the daily weight gain, μ was a general level, τ_i denoted the treatment effect, $x_{l(k(j))}$ the weight at weaning (time 1), ζ the effect of this weight, γ_m the gender of the animal, G_j the group (weekly batches of farrowings), $S_{k(j)}$ the sow, and the last term denoted the interaction between treatment and sow. The last 3 terms were all treated as random components. In addition to these, we also had a random term being normally distributed. The weight gain in the grower-finisher period was analyzed by the same model.

The weight at the last weighing was analyzed by the model:

$$w_{ijkl} = \mu + \tau_i + \zeta \cdot x_{l(k(j))} + \xi \cdot v_{l(k(j))} + G_j + S_{k(j)}$$

where w was the weight at the last weighing (time 3), τ_i the treatment effect, x the age of the animal, ζ the effect of age, ξ the effect of the weight at weaning, G_j the effect of the group, and $S_{k(j)}$ the random component originating from the sow. Not given in the formula above is the random term of the residuals following a normal distribution.

The analyses of weight gain in herd B were performed by similar models as in herd A, but including the adjuvant group and excluding gender and parity. The weight at the last weighing was analyzed by the same model as used in herd A.

The change in OD-values from 1st vaccination to the end of the nurs-

ery period was analyzed in a logistic regression model. The final model was:

$$OD_{ijkl} = \mu + \tau_i + S_{k(ij)}$$

where τ_i referred to treatment group, while $S_{k(ij)}$ was related to the sow and allowing this to interact with group number (j) and treatment. In the initial models gender, age, and parity were included as well, but had no significant influence. In both herds the final, random, component in the models was assumed to follow a normal distribution.

All computations were carried out using the GLMM and REML procedures of the statistical package Genstat (13).

RESULTS

MORTALITY

The effects of vaccination on mortality, average daily weight gain, and serology are summarized in Table II. The mortality data from the 2 herds were analyzed together in the same analysis. Vaccination and weight at weaning had a statistically significant effect on the post weaning mortality due to ED. The odds ratio for the effect of the vaccine on mortality was 0.039 [confidence interval (CI) 0.0089–0.1726], indicating that a 5% mortality due to ED in a herd can be expected to be reduced to 0.2% by vaccination. The relationship between mortality, treatment, and weight at weaning is illustrated in Figure 1. The figure shows that vaccination almost eliminated the risk of dying of ED and that the risk decreased with increasing weight.

AVERAGE DAILY WEIGHT GAIN

The effects of vaccination on growth were analyzed separately for the 2 herds. In both herds weight at weaning was included in the model. The gender of the pigs was included in the model used to analyze data from herd A but not from herd B. In both herds statistically significant increases in ADG were observed in the vaccinated groups in the nurseries. In herd A there was a negative effect of vaccination on the estimated ADG in grower and finisher units, while in herd B the effect of vaccination was similar to the adjuvant effect, indicating no effect. However none of the results differed significantly from the control groups. The results are shown in Table III.

To assess the overall effect of vaccination on growth, the estimated weight of the pigs at the last weighing was calculated in a model including treatment, weight at weaning, and age of the pigs at last weighing. In herd A, the last weighing took place 82 days post weaning (p.w.), on average, and in herd B on average 127 days p.w. In herd B, vaccination had a statistically significant effect on the estimated weight at the last weighing (time 3). The effect of vaccination on herd A and injection of adjuvant without antigen (the adjuvant group) in herd B on weight at the last weighing were not statistically significant (Table IV).
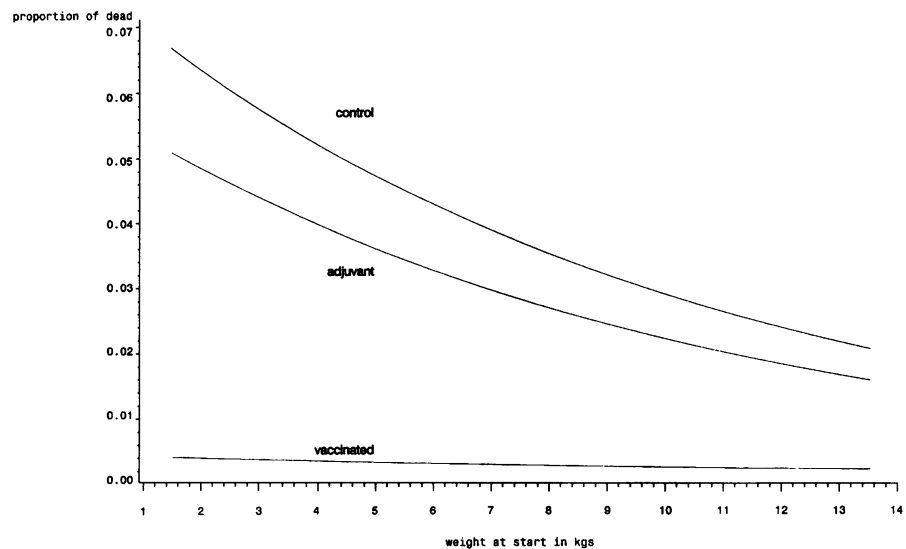

Figure 1. Graphic illustration of the relation between mortality, treatment and weight of pigs.

TABLE III. Difference in estimated average daily gain between vaccine/adjuvant and control groups in nursery and grower-finisher units

	Herd A	Herd B	
	Vaccine	Vaccine	Adjuvant
Nursery	24 (3–45)[a]	78 (41–115)	4 (−34–42)
Grower-finisher units	−10 (−33–13)	25 (−12–63)	27 (−11–65)

[a] x̄ g/d (95% CI)

TABLE IV. Difference in estimated weight at time 3 between vaccine/adjuvant and control groups in the grower-finisher units

Herd A	Herd B	
Vaccine	Vaccine	Adjuvant
0.63 (−0.82–2.10)[a]	4.5 (1.40–8.9)	1.0 (−1.98–5.38)

[a] x̄ kg (95% CI)

PATHOLOGICAL AND MICROBIOLOGICAL EXAMINATIONS

The diagnosis of ED was based on the isolation of F18+, VT2e+ E. coli O139. However, in herd B, 9 pigs from which F18+, VT2e+ E. coli O139 could not be isolated, showed some macroscopic lesions indicating ED as probable cause of death. The lesions observed in these pigs were enlarged congested mesenteric lymph nodes, fibrin strands, and slightly increased serous fluid in the peritoneal cavity, and further, in some pigs mild edema in the mesocolon. Due to the lack of microbiological confirmation of ED, all these pigs were categorized as 'death due to other causes' (Table II). The microbiological findings in these pigs were hemolytic or non-hemolytic, nontypable E. coli and some E. coli O141ac, all lacking the genes for fim-

briae and toxins that were tested for by PCR. None of the vaccinated pigs in herd B showed macroscopic lesions consistent with ED. In herd B hemolytic E. coli O149 which lacked verotoxin genes were isolated from 10 pigs diagnosed as "death due to other causes." These pigs were distributed in the 3 groups: 2 in vaccine, 3 in adjuvant, and 5 in the control group. In herd A none of the dead pigs showed discrepancies between macroscopic and microbiological findings.

SEROLOGICAL EVALUATION

The effect of vaccination on the serum level of VT2e-specific antibodies was assessed by the rise in OD-values in the indirect ELISA from the first vaccination until the end of the nursery period compared to the controls (Table II and Figure 2). In the final statistical model, treatments and sows were included. In herd B

gender and parity were not included in the initial model. The vaccination had a significant effect on the level of OD-values in both herds. The rise in OD-values due to vaccination was 1.38 (CI 0.95–1.81) and 1.16 (CI 0.92–1.40) in herds A and B, respectively. In herd B the injection of adjuvant without antigen had no significant effect on the OD-values.

DISCUSSION

The results of this study indicate that vaccination with purified VT2e-toxoid can prevent ED in pigs. These results are consistent with findings in experimental models (5,9). Although the treatment was performed on a limited number of pigs and the mortality due to ED was only 6.3% in the control group in herd A and 7%/10.6% in the adjuvant/control group in herd B

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herd A
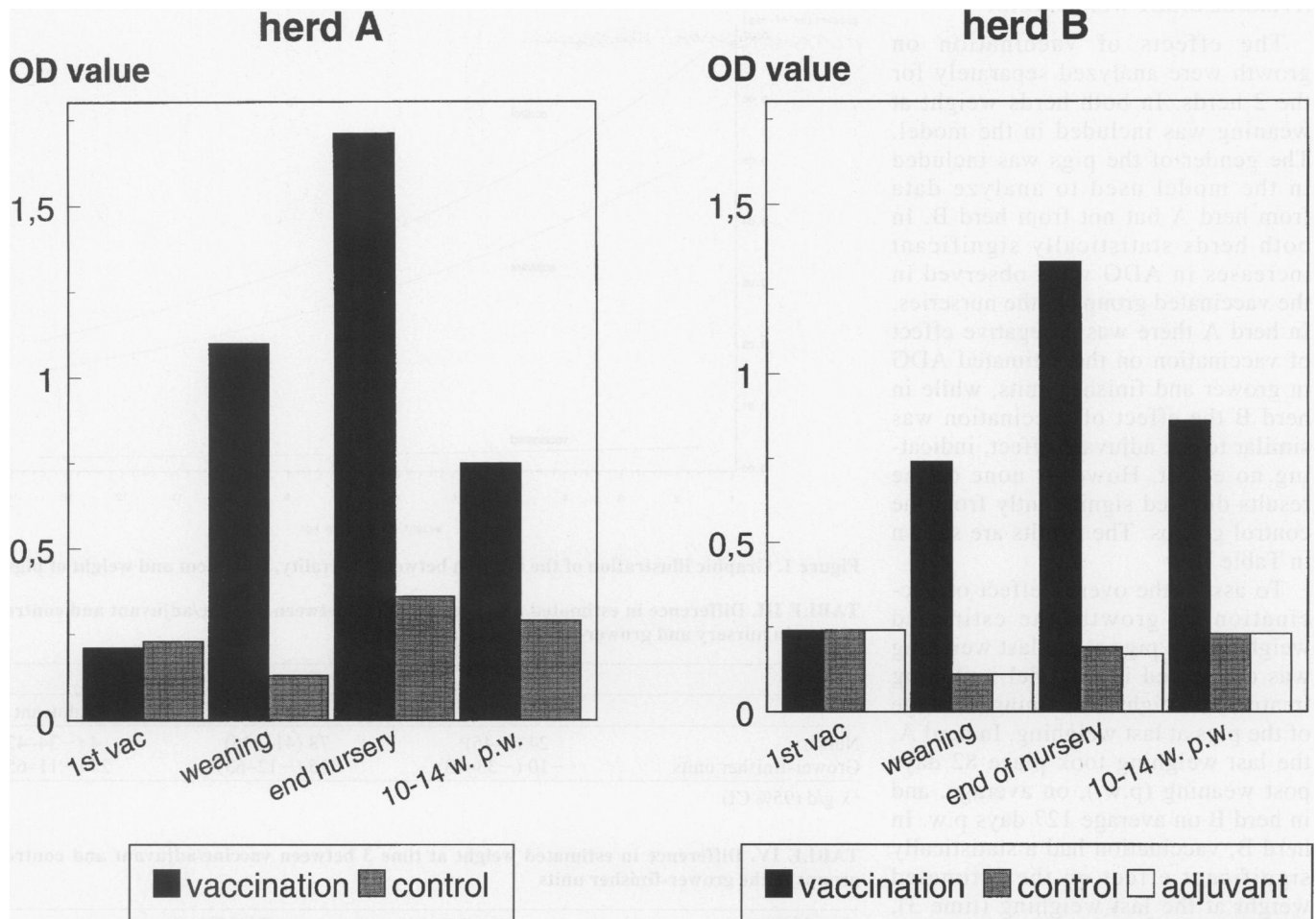
OD value

herd B

OD value

Figure 2. Level of antibodies specific to VT2e in the different treatment groups in herds A and B measured as OD-values in the indirect ELISA at the first vaccination, at weaning, at the end of the nursery period and at 10–14 wk post weaning (p.w.).

during the trial, the effects of vaccination were substantial and it was possible to demonstrate a statistically significant effect on mortality caused by ED.

From Figure 1 it appears that the mortality after weaning decreased with weight at weaning, which were in contrast with the clinical impression, that the largest "best doing" pigs are the ones most susceptible to edema disease. The reason for this was not clarified, but it might have been caused by the fact that the normal cross-fostering after birth was not performed during the trial in herd A. This resulted in some abnormal small pigs in the largest litters in this herd.

In experimental models of ED, diagnosis has been based on histopathological investigations for vascular necrosis (5,14). In the present study some dead piglets were frozen before shipping but others had undergone autolysis prior to their receipt in the laboratory. We decided to define

"death due to edema disease" as cases with growth of hemolytic F18+, VT2e+ *E. coli* O139 on culture from the intestine, since optimal material for histopathological investigation could not be obtained in all cases and typical pathological lesions of ED are not always evident at the time of examination.

ED-causing *E. coli* isolates are often not recovered from pigs with clinical signs and gross lesions typical of ED (4, personal observations). In experimental studies on reproduction of ED in pigs Smith and Halls (15) demonstrated that signs of ED were not apparent until some time after the massive intestinal colonization with the challenge strain of *E. coli*, when the numbers of the bacteria in the intestine were relatively low. This observation in ED is similar to the delayed development of verotoxigenic *E. coli* induced hemolytic uremic syndrome in human beings, a disease which, like ED, appears to be a sys-

temic manifestation of absorbed verotoxin (16). Thus, it is likely that some or all of the dead pigs with macroscopic findings indicative of ED did indeed have ED. In this case the effects of vaccination were even more impressive than indicated by our conservative estimation of deaths due to ED (Table II). The isolation of F18+, VT2e+ *E. coli* O139 might have been improved by culturing from mesenteric lymph nodes (17).

There was no difference in mortality due to other causes between the vaccination group and the control group in herd A. However, in herd B the mortality due to other causes was lower in the vaccine group than in the control group and the adjuvant group, which might be explained by some cases of ED possibly not being diagnosed microbiologically, as mentioned above. Some nonspecific protection against death due to other causes could also explain this difference. Nonspecific protection and the fact

that other serotypes of *E. coli* may produce the same critical virulence factor VT2e make this toxoid vaccine a possible candidate for testing in herds that have problems with post weaning mortality due to *E. coli* O138 and other serotypes carrying the VT2e gene.

In herds A and B, the ADG values in the nurseries were approximately 10% and 25% higher, respectively, in the vaccinated group than the control group and the adjuvant group. This is consistent with findings in previous studies (5,9). There was no effect of vaccination on growth in the grower-finisher period. Discrepancies in the overall effect on estimated growth in the grower-finisher units in the 2 herds might be explained either by herd differences or by random variation. These findings suggest that pigs whose growth rates are suppressed in the early phases after ED are able to recover and gain satisfactorily in the grower-finisher period.

The levels of antibodies to VT2e before the first vaccination were similar in all groups in both herds, indicating that the immunological status in relation to the toxin responsible for the disease was uniform. In herd A, the serological response following vaccination was better than in herd B, which might be due to herd differences in the ability to respond serologically. It is noteworthy that the lower serological response in herd B still afforded protection against ED. Previous studies used toxin neutralizing titres to evaluate the antibody response. Whereas such titres are a more direct measure of effectiveness of the antibodies, the ELISA is a much more convenient assay especially for large numbers of samples. This study provides data on ELISA measurements of sera known to be protective.

In conclusion, the effects of vaccination of pigs with the VT2e-toxoid vaccine used in this study were: I) induction of protective immunity against ED caused by infection with F18+, VT2e+ *E. coli* O139, seen as an almost total elimination of mortality due to the disease, II) a statistically significant increase in ADG in the nursery period, but no effect on ADG in the grower-finisher period, and III) a statistically significant rise in antibodies to VT2e in the vaccinated pigs compared to the pigs in the control and adjuvant groups.

ACKNOWLEDGMENTS

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